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Morphological and Physiological Aspects of Coloration in the Land Crab *Gecarcinus lateralis* (Fréminville, 1835)

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The first author dedicates this paper to the memory of Dorothy E. Bliss, a wonderfully patient mentor, beloved colleague, and dedicated scientist. She loved to learn about crustaceans, and, most importantly, she delighted in the sharing of this knowledge. Dorothy was and shall continue to be an inspiration to many who become interested in the study of shrimps, lobsters, and crabs.

ABSTRACT

A comparison of coloration in *Gecarcinus lateralis* (Fréminville, 1835) from Bimini, Bahamas, and from the Bermudas is made on the basis of morphological and physiological observations. In terms of dark purple shell pigmentation, no notable differences are seen between crabs from the two regions. Bermuda crabs, however, do possess a characteristically brighter reddish-orange coloration than do Bimini crabs, particularly in the posterolateral parts of the carapace. Additionally, Bermuda specimens have many more erythrophores and fewer melanophores than Bimini ones, further enhancing the overall difference in this aspect of their chromogenic coloration. We hypothesize that this color variation may be attributable to the local selection pressures of the respective habitats.

Physiologically, we know of no differences between crabs from Bimini and those from Bermuda; i.e., chromomotor responses are the same in each. Furthermore, several neurotransmitters and opioid substances are found to influence the chromatophoral color change mechanism in *G. lateralis*. These are discussed with reference to possible mode of action. Also, the effects of these substances on the color change mechanisms are compared to those in other species.

INTRODUCTION

For the past 25–30 years, the land crab *Gecarcinus lateralis* (Fréminville, 1835) has been used extensively in physiological and biochemical investigations. Of stable temperament, the crab has proved easy to maintain in the laboratory and resistant to trauma, surgical or otherwise. When kept in community tanks of 10 to 20 individuals accord-

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ing to size, the crabs may fail to molt for many months. If then the crabs are given the privacy of individual containers, along with favorable conditions of illumination, temperature, and moisture, a premolt phase followed by ecdysis usually takes place. The environmental conditions that induce molting in the laboratory are similar to conditions at the bottom of a crab's burrow, where darkness, dampness, relative warmth, and privacy prevail. In nature, *G. lateralis* sheds its old shell and hardens its new one within the burrow. Here, the crab's chances of surviving the vulnerable soft-shell stage are maximal.

Synchrony of molting with favorable environmental factors seems to be effected via the neurosecretory system. Many, possibly most, of the neurosecretory cells that produce and release molt-inhibiting factor appear to reside in the eyestalk ganglia of the crab. Bilateral eyestalk removal (destalking) can induce molting and ecdysis even more rapidly and effectively than do favorable environmental factors. Reimplantation of eyestalk (optic) ganglia (Sandeman, 1982) into the thoracic musculature of the destalked crab delays molting and ecdysis.

With such a responsive and adaptable animal and with such convenient techniques for controlling its growth and molting, a number of biologists have found the crab useful in their investigations. Thus, *G. lateralis* has been used for studies of limb regeneration and molting (Bliss, 1956; Bliss and Boyer, 1964; Bliss and Hopkins, 1974; Hopkins et al., 1979; Skinner, 1962; Tchernigovtzeff, 1972), osmotic and ionic regulation, water uptake, retention, and translocation (Bliss et al., 1966; Mason, 1970; Mantel, 1968), blood pigments and blood coagulation (Stutman and Dolliver, 1968; Mantel et al., 1975), and nucleic acids (Holland and Skinner, 1977). Klaassen (1975) and Bliss et al. (1978) reported observations of *G. lateralis* in the field, where the crab displays remarkable behavioral adaptations for terrestrial life.

For many years, we received living crabs from Bermuda and Bimini in the Bahamas for use in our studies. We have also used crabs from Boca Raton, Florida. These populations differ in color; i.e., one can readily recognize a Bermuda crab, a Bimini crab, and often a Florida crab. From the outset, our interest was awakened by these differences. Over the years, we maintained records of coloration, pigment patterns, chromatophore responses, and so on, for crabs from Bermuda and Bimini. In the first part of this report, we present background information on classification and distribution of *Gecarcinus lateralis,* followed by our observations on morphological aspects of coloration; in the second part we discuss the physiological aspects of coloration.

Acknowledgments

Our appreciation goes to students and colleagues who made important contributions to this study. Mr. Christopher Ray deserves credit for initiating experiments on *Gecarcinus lateralis* that led to further investigations on color change in this species. We thank Dr. Linda H. Mantel for critically reading this manuscript and providing valuable suggestions, and for her continued encouragement throughout the study. Our thanks also to Dr. K. Ranga Rao for reviewing the manuscript and for thoughtful and helpful suggestions.

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CLASSIFICATION AND NOMENCLATURE

The land crab *Gecarcinus lateralis* (Fréminville, 1835) is a reptantian decapod crustacean of the section Brachyura, subsection Brachygnatha, superfamily Brachyrhyncha, and family Gecarcinidae (classification according to Waterman and Chace, 1960). Rathbun (1918) provided diagnostic characters for the Gecarcinidae, as well as a key to the American genera of this family. She also gave a description of the genus *Gecarcinus* and the species *G. lateralis* (see also Chace and Hobbs, 1969).

In addition to species of the genus *Gecarcinus,* the family Gecarcinidae includes those of the genera *Cardisoma* and *Ucides. Cardisoma guanhumi* is a large land crab found commonly in southern Florida and the West Indies; it is marketed in some parts of the

West Indies. *Ucides cordatus* is an inhabitant of mangrove swamps in the same areas. Another American species of *Gecarcinus,* namely *G. ruricola,* is known as the black or blue land crab and is commonly available in West Indian markets.

With increasing scientific interest in *Gecarcinus lateralis,* which has often been called the purple land crab, attention has recently been given to its nomenclature. In 1970, Türkay designated the crab as *Gecarcinus* (*Gecarcinus*) *lateralis lateralis* to distinguish it from *G.* (*G.*) *lateralis quadratus* in the eastern Pacific. This crab had formerly been known as *G. quadratus* Saussure. Subsequently, Türkay (1973) concluded that *G.* (*G.*) *lateralis lateralis* and *G.* (*G.*) *lateralis quadratus* are not separable as subspecies. The name thus becomes simply *G.* (*G.*) *lateralis* (Fréminville).

Bott (1955) had also decided that *G. lateralis* and *G. quadratus* are not separate species. Rathbun (1918) had stated that a notch at the distal edge of the merus of the third maxillipeds distinguished *G. lateralis.* Both Türkay (1973) and Klaassen (1975) showed that all transitional forms of notch, from a deep concavity to a slight depression, can be found; or the notch may even be absent. Thus, the notch has little value as a distinguishing characteristic. Other research (Klaassen, 1975) has shown that the Atlantic and Pacific forms of *Gecarcinus* are not identical. In this paper, we are concerned solely with the Atlantic form, for which we use the name *Gecarcinus lateralis* (Fréminville).

OCCURRENCE AND DISTRIBUTION

The range of *Gecarcinus lateralis* extends from the Bermuda Islands through the Bahama Islands, the Florida Keys, and the East and West Indies, along the Atlantic coast of Central America to Colombia, Venezuela, and French Guiana, and to Ascension Island (Rathbun, 1918), although the last record is being questioned (Chace, personal commun.). A dense population of *G. lateralis* has also been reported on the Florida mainland at Boca Raton (Bliss et al., 1978). This crab is also found at South Padre Island, Texas (Ray, 1967; Britton, 1976).

In areas where *G. lateralis* has been studied, it has always been found above the high tide line. In Bermuda, the crabs may be dug out of burrows on the upper sandy beach, and on high grass- and weed-covered areas extending well inland (Weitzman, 1963). In Bimini, the crabs do not burrow on the upper sandy beach, but at the edge of the thick grass bordering the beach and extending inland through the grassy areas (Bliss, 1963). In both Bermuda and Bimini, the burrows of the ghost crab, *Ocypode quadrata,* occur from the shore-side border of the area occupied by *Gecarcinus* down through the tidal zone to the ocean. There is no overlap in the niches of the two species. The large land crab *Cardisoma guanhumi* is restricted to places where it can dig down to seawater; in local areas occupied by both *Cardisoma* and *Gecarcinus,* the two crabs remain segregated.

Observations reported by Klaassen (1975) indicate that in northern Colombia the land crab *G. lateralis* inhabits loamy soil in coconut tree groves, the upper portion of vegetation-covered stationary dunes, soil-covered cliff walls of fossiliferous coral rising steeply from the water, high, dirt-covered, but otherwise exposed rocks, and cultivated gardens. On the island of Dominica, *G. lateralis* can be found at elevations of about 300 m (Chace and Hobbs, 1969).

At Sabal Point, Boca Raton, Florida, *G. lateralis* occurred densely in a heavily canopied dune area and somewhat less densely on a more exposed ridge of sand shaded by Australian pines (*Casuarina equisetifolia*). In more exposed areas of the vine-covered upper beach, there were fewer burrows (Bliss et al., 1978). Sadly, this local population of *G. lateralis* is now extinct due to commercial development in the region.

The variety of habitats in which *G. lateralis* prospers is testimony to its adaptability as a terrestrial animal. Morphological, physiological, and behavioral adaptations have facilitated the crab's invasion of the various terrestrial areas. Its coloration, too, may well have assisted in this process. This subject is discussed in subsequent pages.

MORPHOLOGICAL ASPECTS OF COLORATION

Distribution of Shell Pigments

A brief description of the coloration of *Gecarcinus lateralis* appears in Rathbun (1918:

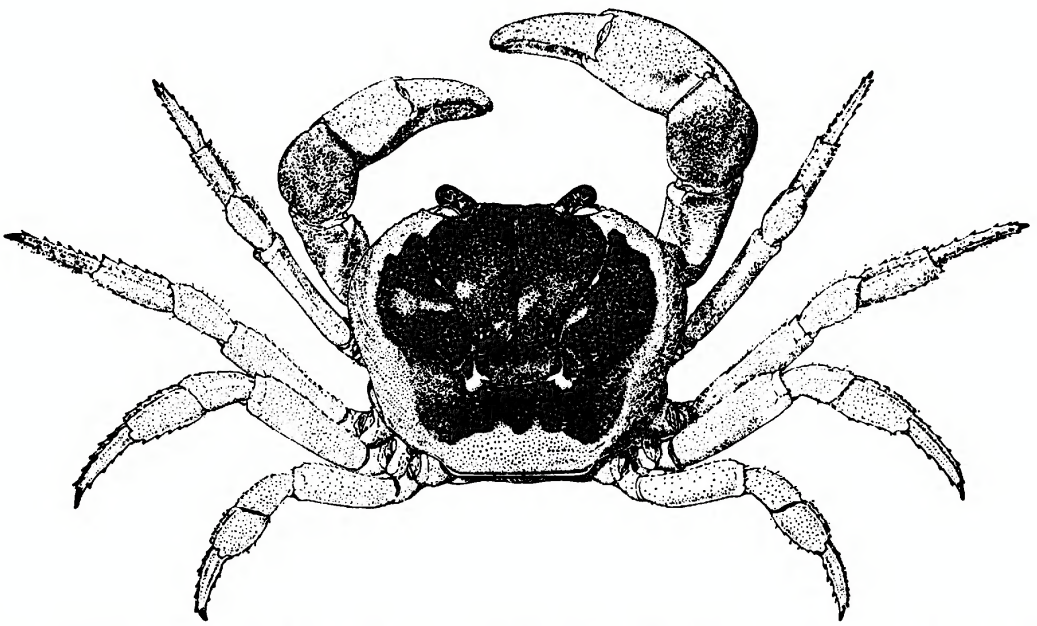


Fig. 1. *Gecarcinus lateralis* from Dominica (from Chace and Hobbs, 1969, with permission from the authors).

356). Considerably more details are given by Chace and Hobbs (1969: 198–199) for *G. lateralis* of Dominica. With these authors' permission, an excerpt of this description is reproduced below, as is their figure 65 (fig. 1 of this paper).

> COLOR IN LIFE—Carapace with large central area of black extending from front posteriorly and posterolaterally to posterior fifth with pair of small white or cream spots along cervical groove between branchial and protogastric regions, smaller pale pair in posterior portion of mesogastric-protogastric groove, and conspicuous pair in anterolateral portion of gastric region. Small irregular cream area with white center immediately posterior to lateral portion of each orbit; anterolateral areas scarlet purple bearing red oblique lines, and fading posteriorly along lateral area to scarlet and finally to orange; irregular band of latter extend across posterior portion of carapace.

The authors also describe coloration patterns on the chelipeds, walking legs, and ventral surface of the body. Much of the coloration described by Chace and Hobbs is attributable to pigments deposited within the shell. Using *G. lateralis* from Bermuda and Bimini, we recognize two classes of patterns in which the pigments of the carapace are arranged. In class A (fig. 2), dark purple pigment extends completely across the carapace and down over the sides (branchiostegites). Variations in color are restricted to a relatively small, lightly colored area on the posterior part of the carapace. Here coloration is determined chiefly by the extent to which pigment within the epithelial chromatophores is concentrated or dispersed. The size of the lightly colored area on the posterior part of the carapace ranges from very small to fairly sizable (fig. 2, A1–A6).

In class B, dark purple pigment initially extends across the anterior part of the carapace (fig. 3, B1). In B2 through B5, generally the lateral parts of the carapace are paler. In B6, only the gastric region remains dark purple. Purple pigment seems to have been deposited over plum-colored pigment, since the plum color is visible when the purple pigment is removed by rubbing with fine sandpaper.

In class B as in class A, the carapace is only lightly pigmented posteriorly, and this coloration may depend primarily on the extent of dispersion of pigments within the chromatophores. In both classes, dark purple pigment is present to some degree on the pereiopods (walking legs), but again depends primarily on the state of dispersion. In the

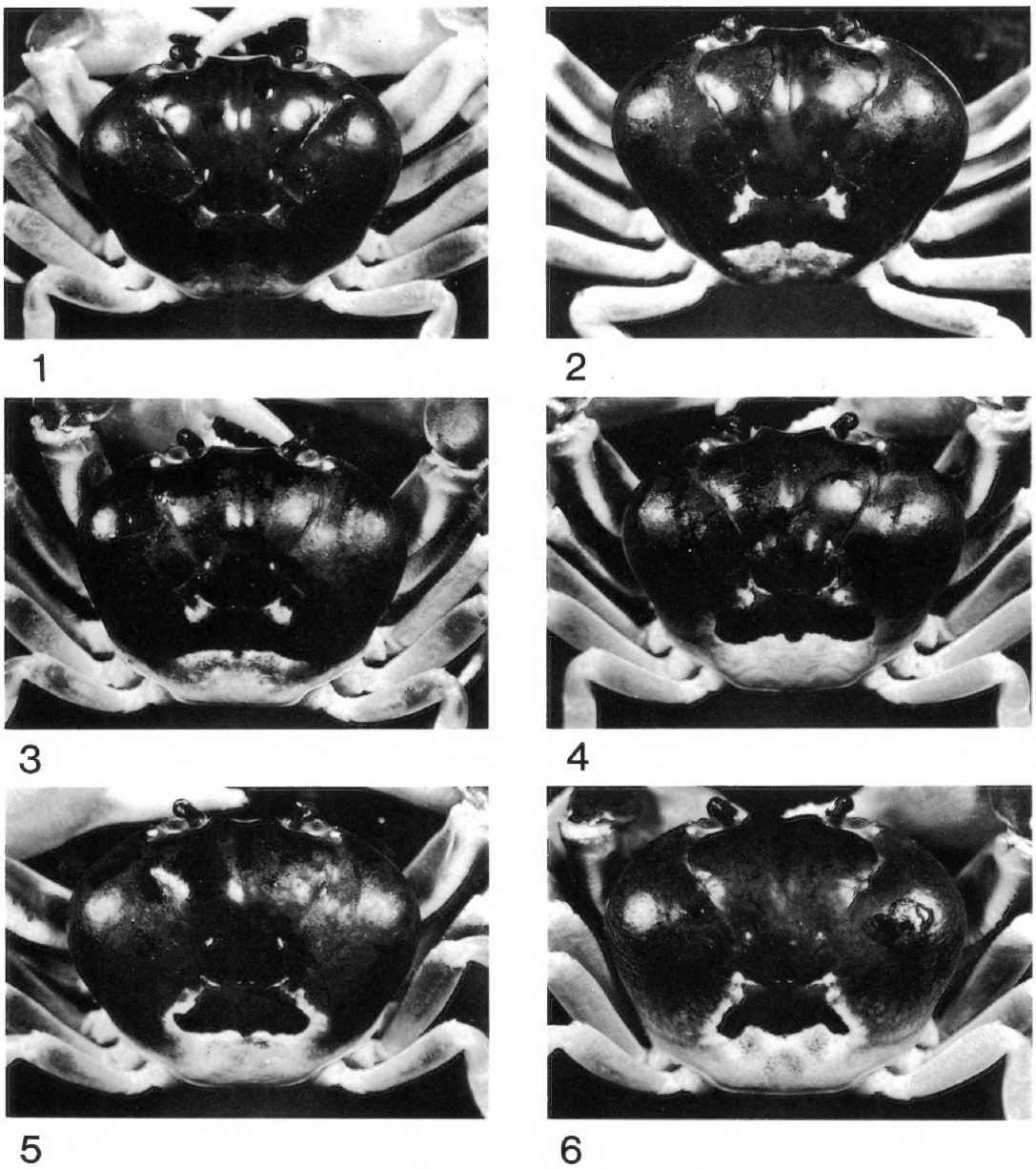


Fig. 2. Class A shell pigment patterns.

claws, however, considerably more shell pigment is present and, to a large extent, this determines their color.

Pigment patterns of class B appear much more frequently than do those of class A in crabs from both Bermuda and Bimini. In a sample of 844 male crabs from Bermuda, there were approximately seven times as many pigment patterns of class B (or 87% of total) as of class A (13% of total; fig. 4). Among 385 female crabs from Bermuda, there were about 16 times as many pigment patterns of class B (94% of total) as of class A (6% of total). Similarly, among 375 male crabs from Bimini, there were about 24 times as many pigment patterns of class B (96% of total) as of class A (4% of total); and among 141 female crabs, there were 65 times as many of class B (98% of total) as of class A (2% of total; fig. 4). When pigment patterns of class

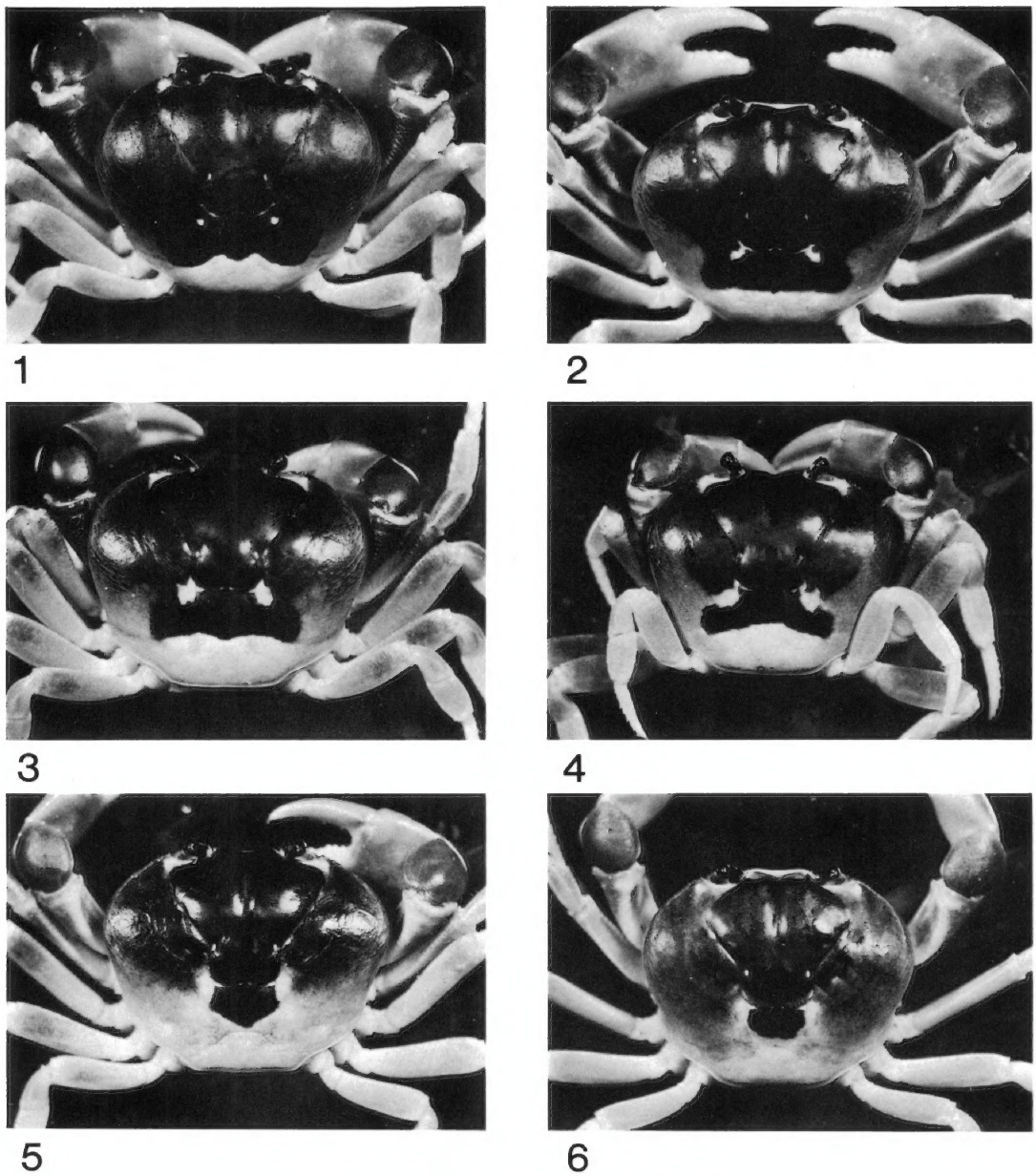


Fig. 3. Class B shell pigment patterns.

A are present, they occur predominantly in large male and female crabs from Bermuda. This pattern is comparatively rare in crabs from Bimini.

Among pigment patterns of class B, the frequency of occurrence of the various types is consistent (fig. 5). Thus, among male crabs from Bermuda, type B4 predominates in all four size classes. (For convenience, crabs are separated into four size classes according to carapace width: I, 1.8–3.7 cm; II, 3.8–4.6 cm; III, 4.7–5.2 cm; IV, over 5.2 cm.) Within size

→

Fig. 5. Frequency of occurrence of types 1–6 of Class B shell pigment patterns according to size classes (I–IV) among male (♂) and female (♀) crabs from Bimini and Bermuda. Size classes are according to carapace width: I, 1.8–3.7 cm; II, 3.8–4.6 cm; III, 4.7–5.2 cm; IV, >5.2 cm.

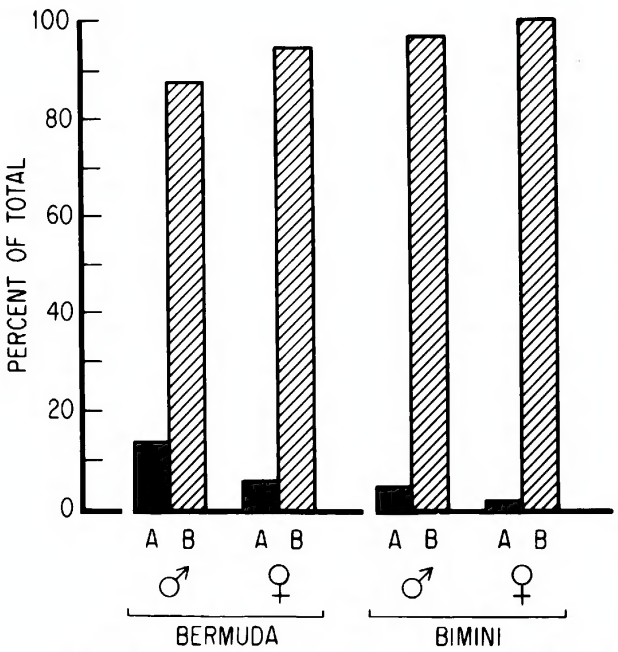


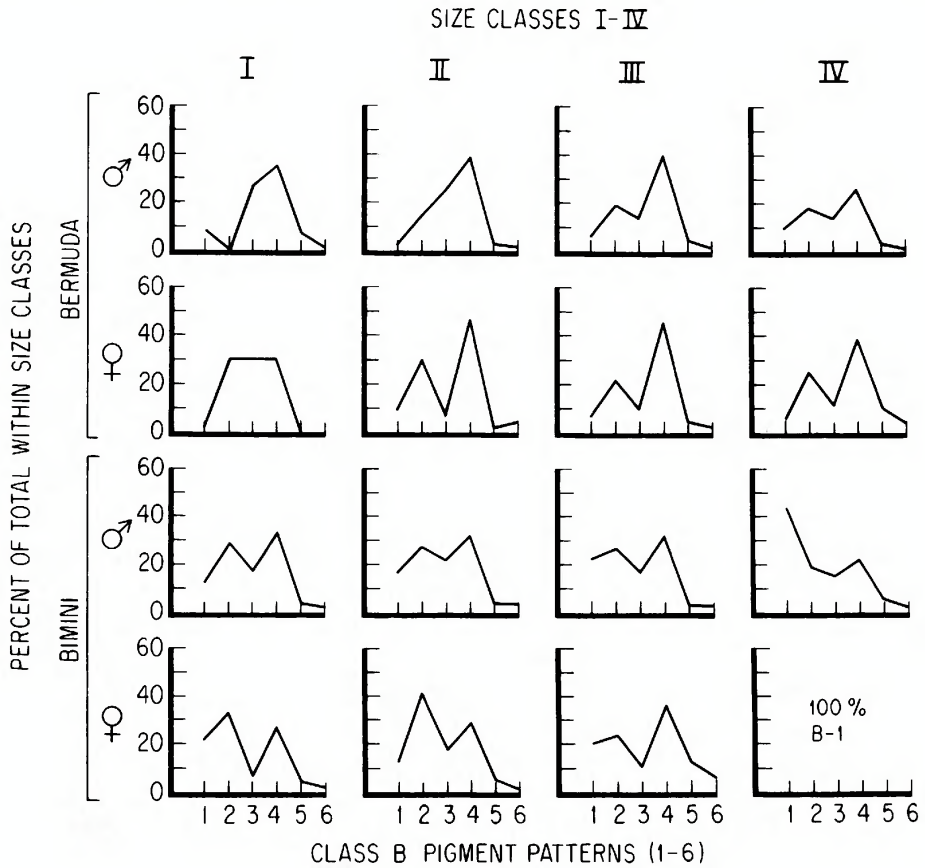
Fig. 4. Distribution of Class A and Class B shell pigment patterns among male (♂) and female (♀) crabs from Bimini and Bermuda.

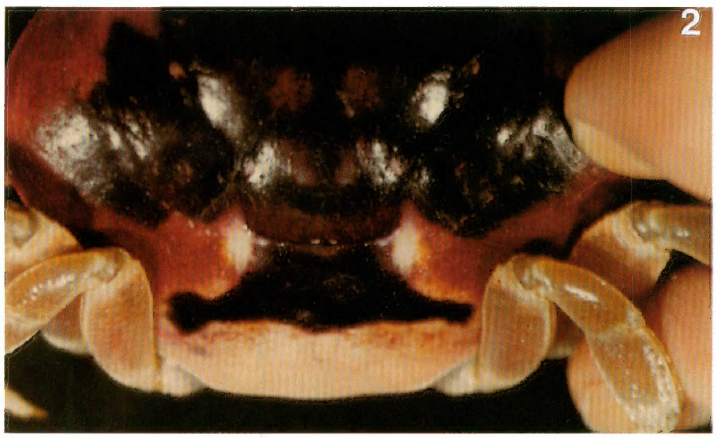
classes III and IV, type B2 appears with next greatest frequency. A similar pattern of distribution exists among female crabs from Bermuda. As for male and female crabs from Bimini, for size classes I through III there is a similar frequency distribution.

These observations suggest that, in terms of the distribution of shell pigments, there is no characteristic difference between crabs from Bermuda and those from Bimini.

Variation and Diurnal Change in Coloration

Although shell pigment patterns of *G. lateralis* from Bermuda and Bimini are similar, this is not true of the overall coloration. In crabs from Bermuda, the legs and posterior part of the carapace are variously bright orange, red, yellow, or light shade of pink, but seldom gray (pl. 1, fig. 1; pl. 2, fig. 1); in crabs from Bimini, they are often light to dark gray (pl. 1, fig. 2; pl. 1, fig. 3). This difference in coloration appears to be related in part to the





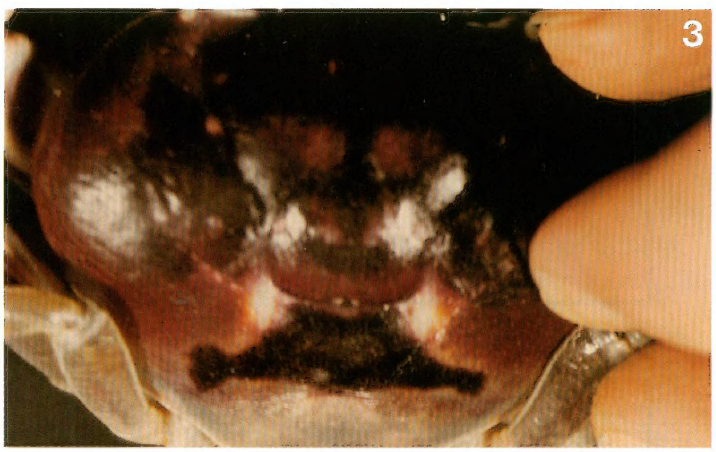


Plate 1. Comparison of overall coloration of a Bermuda crab (fig. 1) to a Bimini crab (figs. 2, 3; same crab at different chromatophoral states).

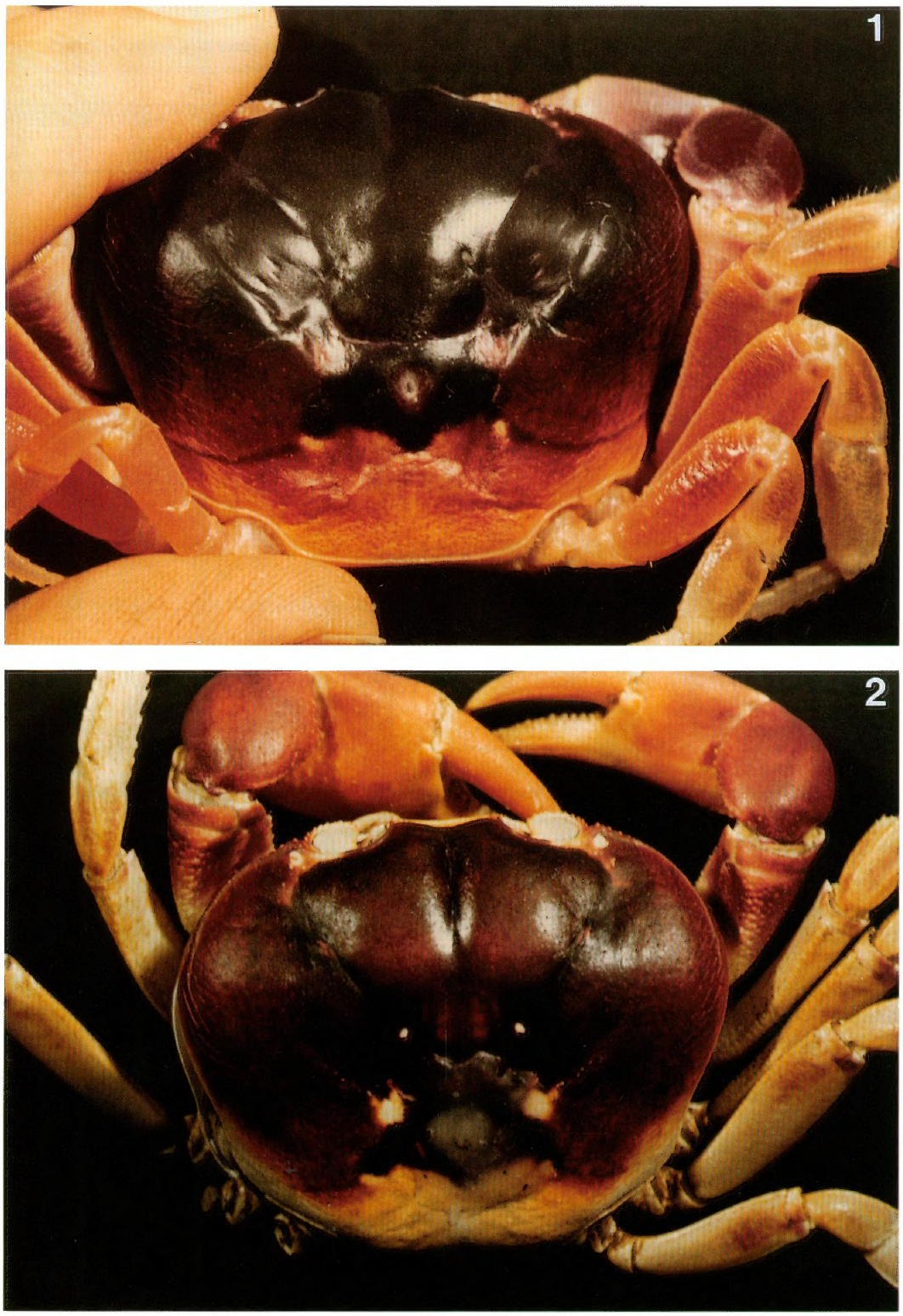


Plate 2. Comparison of the coloration of a newly molted Bermuda crab (fig. 1) with the coloration of its cast shell (fig. 2).

TABLE 1

Observations of Diurnal Rhythms in Coloration in *Gecarcinus lateralis* from Bimini[a]

	Day 1			Day 2				Day 3		Day 4	
	P.M.			A.M.			P.M.	A.M.	P.M.	A.M.	P.M.
State of coloration	2:30	7:00[b]	10:15	5:15	7:05[c]	9:15	7:50[c]	7:30[c]	6:50[c]	7:45[c]	6:45[b]
Pale orange and white	L	L						L	L	L	L
Cream white with touches of pale orange	F									F	F
Gray with touches or speckles of orange	D J	D F									
Dark gray or bluish gray	H M	H	H J	H J	H J D	H J D	H D	H J	H D	H J	D
White		M	M F L	M F L	F L	M F L	M F L	F	F		M
Pale orange		J	D	D					J		
Grayish-white		F			M			M		M	
Mottled dark gray and orange							J			D	H J
Light gray with orange								D			
White with faint touch of gray									M		

[a] See text for observations on coloration in Bermuda crabs.

[b] Lights turned out immediately after observation was completed. Time indicates when observation was started.

[c] Lights turned on at this time. Observation taken immediately thereafter.

relative number of black and red chromatophores within the epithelium and the degree of dispersion of pigment within the chromatophores. Yet to some extent it also results from the amount and kind of pigment within the exoskeleton. For one crab from Bermuda, the extent to which shell pigments determined overall coloration is apparent from plate 2, which shows a newly molted crab (fig. 1) and the cast shell (fig. 2). The coloration of the cast is attributable to shell pigment; the coloration of the newly molted soft crab is determined by both shell pigment and pigment within epithelial chromatophores. In a soft crab, one can see chromatophoral pigments with remarkable clarity.

Differences in coloration between crabs from Bimini and those from Bermuda are not due to diet. In the laboratory, after being maintained for many months on a diet of peanuts, carrots, lettuce, egg shells, and Purina dog chow, crabs from Bermuda seemed unchanged in coloration. Crabs from Bimini sometimes became less gray but never brightly colored like the individuals from Bermuda.

To obtain some idea of the variation in coloration of the posterior part of the carapace that can occur during consecutive 24-hour periods, observations were made for seven days on eight crabs from Bermuda and six crabs from Bimini. The time of observation was as early as 5:15 A.M. on one occasion and as late as 10:15 P.M. on another. Most observations, however, took place between 7:00 and 9:00 A.M. and between 6:45 and 7:30 P.M. Each crab was identified by letter and kept in a quart-size jar or plastic box (30 × 15 × 8.75 cm) which was covered approximately 85 percent with black Contac paper during the period of observation. The

Table 1—*Extended*

Day 5		Day 6	Day 7	
A.M.	P.M.	A.M.	A.M.	P.M.
8:40[c]	7:30[b]	9:25[c]	6:50[b]	8:05[c]
L	L	L	L	L
	F	F		
	D	H		D, H
	H			J
J				
		J	J	
F	M		M, F	
	J			
M		M		
D		D	D	
H			H	
			M	
			F	

time at which the lights were turned on and off was somewhat variable. The temperature was 22 ± 1°C.

Only one crab from Bermuda had melanophores in sufficient numbers or with pigment sufficiently dispersed to darken the posterior part of the carapace. This one crab was light orange and white with speckles of gray or gray with touches or speckles of orange in essentially equal frequency. On one occasion the rear of the carapace of this crab appeared mottled dark gray and orange. It seemed not to matter whether observations were made just after the lights had been turned on, after they were off all night; or just after the lights had been turned off, after they were on all day. Either the largely orange or largely gray coloration could exist under both conditions.

The remaining seven crabs from Bermuda were consistently bright orange with touches of red, yellow, or white. No matter what the time of observation or the length of time that the lights had been on or off, the coloration of these crabs was the same.

A summary of the observations on Bimini crabs appears in table 1. At the left of the table is a list of 10 different states of coloration that were recorded for the posterior part of the carapace. These range from pale orange through various shades of gray or gray with touches of orange to essentially pure white. Three of the crabs were essentially white. One of these (M) became grayish-white during the night, appearing such on five occasions when the lights went on in the morning. The other two (F, L) remained white at all observations. Crab D was pale orange when observed three hours after the lights went out and two hours before they came on during one nighttime period of observation (the crab was illuminated very briefly when actually examined). This same crab was mottled dark gray and orange after the lights had been on all day and dark gray after they had been off all night.

Another crab from Bimini (H) was dark bluish-gray in the morning and evening, and at night for 10 consecutive observations, then gray with touches of orange or mottled dark gray and orange for the remaining five observations.

The sixth crab from Bimini (J) was pale orange (pl. 1, fig. 2) or mottled gray at five nighttime observations after the lights had been on all day and gray (pl. 1, fig. 3) during the night or after the lights had been off all night.

From this limited, short-term experiment, one may conclude that there is no evidence of diurnal rhythmicity in crabs from Bermuda. Seven of the crabs did not change in coloration at all; the eighth changed from orange with speckles of gray to gray with touches or speckles of orange, but either coloration could exist day or night. However, in some crabs from Bimini, there was evidence for diurnal rhythmicity in coloration. Two crabs (D and H) were dark gray after the lights had been off all night and dark gray and orange after the lights had been on all day. Both of these crabs, however, when observed during the night, were sometimes pale orange. So it is not clear just when the shift from orange

to gray occurred. A third crab (M) from Bimini changed from white to grayish-white at night.

This does not necessarily mean, however, that only crabs from Bimini are diurnally rhythmic in coloration. The posterior carapace in crabs from Bermuda is characteristically bright orange, with touches of red, yellow, or white. Much of this coloration may be due to pigments within the shell. If so, these shell pigments may be obscuring diurnal changes in chromatophoral pigments of the underlying epidermis. In crabs from Bimini, the posterior carapace is not heavily impregnated with bright orange and red-orange shell pigments. Instead, it is generally white or pale orange. Changes in the underlying epidermal chromatophores are much more readily visible in crabs from Bimini, therefore, than in crabs from Bermuda.

There is yet a second possibility regarding these differences. The brightly orange or red-orange coloration of the posterior carapace in crabs from Bermuda may be partly due to the presence of many more erythrophores, containing well-dispersed pigment. Added to the considerable orange and red-orange shell pigment, these dispersed erythrophoral pigments would tend to further obscure changes in other pigmentary epithelial cells, such as the melanophores.

Since the posterior carapace in crabs from Bimini is consistently white or pale orange when melanophoral pigments are concentrated, this area would appear to lack heavy orange or red-orange shell pigment. But it may also have fewer erythrophores—and conceivably more melanophores—than does the corresponding area in crabs from Bermuda. In light of this possibility, counts were made of erythrophores and melanophores in crabs from both localities. Results are presented in the following section.

Before leaving the subject of changes in coloration in crabs from Bimini, it should again be noted that all observations described above were made on crabs maintained in individual private containers. On one occasion, an observation on crabs kept in community tanks suggested the possibility of a "community factor" in determining coloration.

A shipment of crabs had arrived from Bimini on August 21, 1964. On September 1, at 3:00 P.M., the second author was starting to examine crabs in two tanks for types of pigment patterns. Although both tanks were on the same tier, one directly above the other, the crabs in one tank were all light-colored, while those in the other tank were all dark gray. Examination of the first 10 dark gray crabs began; by the time the first seven had been examined, the posterior part of the carapace and the legs of the 10 crabs had lightened. During the lightening, the lateral areas of the carapace lightened also, so that the center area that is sharply outlined in crabs of class II was easier to see. Even when the crabs are dark gray, however, the pigment pattern can readily be detected and classified.

As examination continued, more and more crabs from the tank lightened, so that by the time all were returned to the stock tank, most were no longer dark gray. In the tank of crabs that were originally light-colored, most were still so—but a few were now dark gray.

Crabs arriving from Bimini in a storage container tend to be dark gray when first removed; within an hour, however, many, if not most, have lightened. It seems clear that graying and lightening of crabs from Bimini are governed by a complex of factors.

Distribution and Number of Chromatophores

Both erythrophores and melanophores are generally distributed throughout the exoskeleton of *G. lateralis*. Although leucophores and xanthophores are present in other crustaceans, these were not observed in *G. lateralis*. The erythrophores and melanophores are most readily seen on the posterior part of the carapace and on the dorsal surface of the pereiopods. In regions where the exoskeleton is heavily pigmented, these chromatophores can be seen when the shell pigment is removed with fine sandpaper. On the claws, however, few erythrophores and melanophores are visible in the larger individuals (4.0–5.0 cm carapace width), even when the dark purple or red pigment has been removed. Interestingly, a newly molted large crab displays numerous chromatophores on its chelipeds. Young crabs and newly molted older crabs display chromatophores throughout their body; abrasion of the shell is not required for these to be seen.

TABLE 2

Average Number of Chromatophores per mm² in *Gecarcinus lateralis* from Bimini and Bermuda[a]

Region of carapace	Bimini crabs		Bermuda crabs	
	Melanophores	Erythrophores	Melanophores	Erythrophores
Left dorsolateral	17.8 ± 1.5	20.1 ± 1.6	12.2 ± 2.0	30.4 ± 2.7
Cardiac	17.4 ± 1.6	21.7 ± 2.2	11.7 ± 1.9	30.2 ± 2.8
Right dorsolateral	16.9 ± 1.5	22.1 ± 2.1	12.1 ± 1.8	31.6 ± 3.0

[a] Mean ± SE; Bimini crabs, N = 21; Bermuda crabs, N = 13.

On the dorsal surface, chromatophores are fairly evenly distributed over the entire carapace in both Bermuda and Bimini crabs. On the pereiopods, there appears to be a random distribution of chromatophores, except on the propodus, where the melanophores are arranged in a somewhat zig-zag pattern.

On the ventral surface, fewer chromatophores are visible. They occur in greatest concentration around the mouth parts and in the branchial region. Few melanophores but numerous erythrophores can be seen on the abdomen. On the thoracic segments and at the base of the legs, there are almost no chromatophores. Only in the smaller crabs (e.g., around 2.5 cm carapace width) are chromatophores found in these areas.

Erythrophores and melanophores were counted in three selected areas of the posterior part of the carapace (cardiac region and dorsolateral part of the branchiostegites) after these areas had been rubbed down with fine sandpaper. To make the counts, a calibrated grid (area 0.25 mm²) was placed in a 10× eyepiece of a stereomicroscope. In 21 crabs from Bimini and 13 crabs from Bermuda, the number of erythrophores and melanophores per square millimeter was determined for each area; means and standard errors were calculated. These are presented in table 2.

From table 2, it is clear that in crabs from Bermuda, overall there are more erythrophores than in crabs from Bimini. Conversely, in crabs from Bimini, there are many more melanophores than in crabs from Bermuda. These results strengthen the likelihood that the vivid orange and reddish-orange coloration in the posterior carapace of crabs from Bermuda is related not entirely to shell pigment but also to the plentitude of erythrophores in this area. Furthermore, the scarcity of melanophores makes graying of this area difficult. As for crabs from Bimini, the dark gray to light coloration of the posterior portion of the carapace is clearly due to the very numerous melanophores. When the pigment in these melanophores is concentrated, the relatively few erythrophores present cannot provide a brilliant orange or red-orange coloration, only a pale orange in this area.

PHYSIOLOGICAL ASPECTS OF COLORATION

Since the original discoveries by Koller (1925, 1927, 1928), Perkins (1928), and Carlson (1936) that blood-borne substances are responsible for migration of pigments within the chromatophores of certain decapod crustaceans, investigations on the various pigmentary effector systems in Crustacea have concentrated on efforts to determine the source and action of the hormones responsible for pigment migration. Reviews by Fingerman (1970), Bagnara and Hadley (1973), Kleinholz (1970, 1985), and Rao (1985) summarize the information presently available on the neurosecretory products of the central nervous system of Crustacea. In recent years, primary efforts in crustacean endocrinology have focused on the characterization, isolation, purification, and synthesis of various neurohormones, including the chromatophorotropins (see Kleinholz, 1976, 1985; Newcomb et al., 1985; Rao and Riehm, 1988). Lately, the regulation of release of the neurohormones involved with color change has been of particular interest (e.g., Fingerman and Fingerman, 1977; Hanumante and Fingerman, 1982; Fingerman et al., 1981; Martinez, 1986; Martinez et al., 1986; Rao and Fingerman, 1983; Quackenbush and Fingerman, 1984a, 1984b). In his review on the physiology and pharmacology of crustacean

chromatophores, Fingerman (1985) summarized recent studies on the effects of various drugs on color change mechanisms. He also discussed the role that microtubules may play in the translocation of pigment granules.

In this section, we report our findings on the activity, distribution, and characteristics of certain chromactive factors in *G. lateralis* and compare our findings with those reported for other crustaceans.

Materials and Methods

Gecarcinus lateralis (Fréminville, 1835) was obtained from Bermuda and Bimini, Bahamas. Stocks of crabs (15–20 individuals) were housed in glass-walled tanks (60 × 30 × 30 cm) partly covered with Contac paper. The tanks contained either washed beach sand moistened with tap water or a shallow dish with diluted seawater (15 ppt). The photoperiod consisted of 12 hours of light and 12 hours of darkness every 24 hours, and the temperature was 25 ± 1°C. Every one to two weeks, the crabs were fed peanuts, carrots, lettuce, egg shells, and Purina dog chow.

Eyestalkless crabs averaging 5 cm in carapace width were used in all bioassays. These test animals were maintained in individual, partly covered quart jars containing sand moistened with tap water, or in partly covered plastic boxes (30 × 15 × 8.75 cm) containing a Petri dish with diluted seawater (15 ppt). No differences in coloration pattern or responses to injections were noted as a result of housing the test crabs under these two sets of conditions. These test crabs were not fed. However, maintaining them at 17–18°C was found to prolong their premolt condition, thus rendering the crabs usable over a longer period of time and reducing the rate of mortality within test populations.

Proximity to molting was monitored by periodic measurements of regenerating limbs (Bliss and Boyer, 1964; Bliss and Hopkins, 1974). Two to three days prior to destalking, specimens of *G. lateralis* with no limbs missing were induced to autotomize their third walking leg. Every two to three days thereafter, the crabs were weighed and their regenerating limb bud measured with vernier calipers.

Removal of Eyestalks: At the time of destalking the crabs were chilled until sluggish, then their eyestalks were snipped off at the base. Eyestalks that were not to be used immediately for preparations of extracts were frozen for subsequent use. To prevent bleeding, the stubs of the eyestalks were packed with Gelfoam (Absorbable Gelatin Sponge, U.S.P. Upjohn). No destalked crab was used for bioassays until at least 24 hours after destalking.

Preparation of Tissue Extracts: Freshly removed (or recently frozen) eyestalks, supraesophageal ganglia, or thoracic ganglionic mass tissues of *G. lateralis* were extracted by grinding them in a chilled mortar containing either 1 ml seawater, 1–5 ml distilled demineralized water, or 1 ml *Gecarcinus* perfusion fluid (Skinner et al., 1965) with pH adjusted to 7.3. Unless otherwise indicated, all such extracts were heated in a boiling water bath for 10 minutes and their volumes adjusted to 1 ml. The extracts were then centrifuged at 10,000–15,000 × *g* at −4°C for 10 minutes and the supernatant was decanted and subsequently bioassayed.

Bioassay of Tissue Extracts and Staging of Chromatophores: The hormones that control pigment migration within the chromatophores, i.e., red pigment-concentrating hormone (RPCH) and black pigment-dispersing hormone (BPDH) were bioassayed. All crabs used for bioassays were specimens of *G. lateralis* that had been destalked for at least 24 hours. Generally, 6 to 11 crabs were included in each bioassay, with at least one crab serving as a control. This crab was injected with seawater or *Gecarcinus* perfusion fluid. Depending on the nature of the experiment, other controls consisted of two to four crabs that were injected with either seawater, distilled demineralized water, or extracts of leg nerves or leg muscle. During bioassays, each crab was secured with rubber bands to a wooden block (11 × 10 × 1.87 cm) bearing a sponge or paper toweling moistened with tap water.

Crabs were injected (0.05 ml/dose) with extracts or control fluids through the soft arthrodial membrane at the base of the second or third pereiopod. Observations of chromatophores on the posterior portion of the carapace and/or on the dorsal surface of the pereiopods were conducted with a stereo-

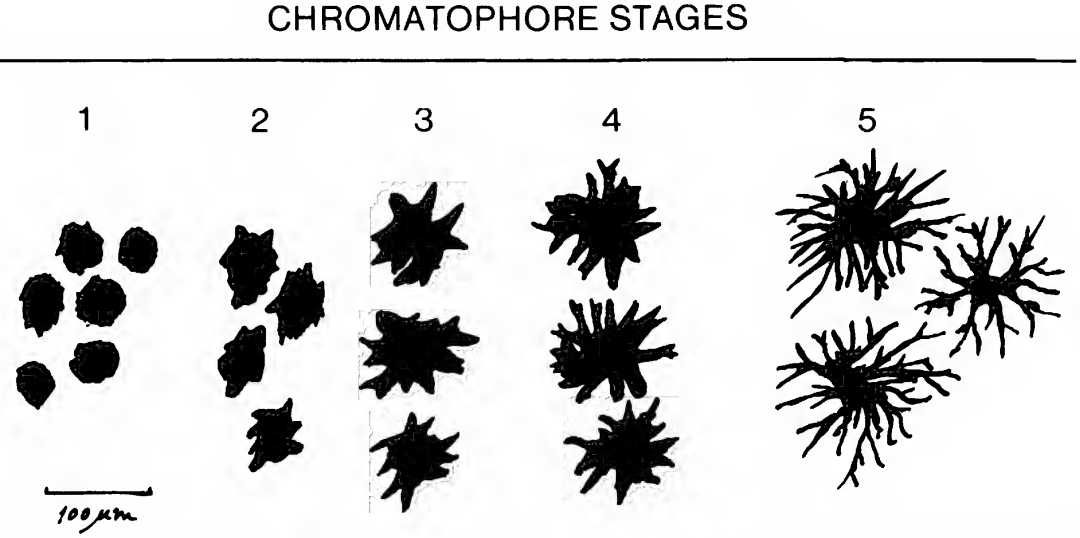


Fig. 6. Stages of chromatophoral pigment migration according to the Hogben and Slome (1931) method.

microscope at low (9×) magnification. With crabs possessing a heavily pigmented shell, it was necessary to gently rub down the rear of the carapace with fine sandpaper to render the chromatophores more visible. Staging of chromatophores was according to the method of Hogben and Slome (1931) in which stage 1 represents maximal concentration and stage 5 maximal dispersion of pigments within the cells; stages 2, 3, and 4 represent intermediate conditions. Staging was conducted 10 minutes after injections and every 15–20 minutes thereafter until the effects of an injection wore off. The various stages of concentration or dispersion of pigments within the cells were then plotted as a function of time. The area under the curves represents the total activity of the extract; i.e., the curves provide an estimate both of the magnitude and the duration of the response.

Sketches of chromatophores illustrating their appearance at various stages of pigment dispersion and concentration are presented in figure 6. Generally, melanophores are easier to observe than erythrophores. At stage 1, the melanophores are quite punctate, whereas erythrophores, although smaller, are slightly stellate. As pigment disperses within each cell type, the processes, extensions, or the stellate nature of the cells become more evident. This is particularly true of the melanophores. However, since erythrophores are more numerous than melanophores, especially among Bermuda crabs (see section on Morphological Aspects), they seem to merge together as the pigment disperses, making it much more difficult to see them as discrete cells. This tends to impart an overall reddish-orange coloration to the animals.

Distribution of Chromatophorotropic Activity Within the Central Nervous System: In order to determine the relative distribution of chromatophorotropic activity within the nervous system of *G. lateralis,* extracts of eyestalks, supraesophageal ganglia (brain), and thoracic ganglionic mass from one donor were prepared with *Gecarcinus* perfusion fluid and bioassayed for RPCH and BPDH activity. From another crab, the right eyestalk, one-half of the supraesophageal ganglia, and one-half of the thoracic ganglionic mass were extracted separately with 0.5 ml perfusion fluid and bioassayed. The remaining halves were combined and similarly extracted and bioassayed. The concentration of each extract was adjusted so that the number of central nervous system organ-equivalents in each dose was proportional to the number of organs normally present in a crab. In a second experiment, extracts were prepared from pooled eyestalks, pooled supraesophageal ganglia, and pooled thoracic ganglionic mass tissues of six donor crabs. Each extract was bioassayed separately and in combination with one

of the other extracts. Here too, each one was made up to a specific volume so that the number of nervous system organ-equivalents per dose (0.05 ml) was known.

Factors Affecting Chromatophorotropic Activity of Eyestalk Extract: Chromatophorotropic activity among crustaceans is known to be influenced by several factors such as concentration and type of extracts being injected, pretreatment of extracts (e.g., heat, freezing, fractionation, etc.), and the physiological state of the animals at the time of bioassay (see review by Rao, 1985). Since our test crabs were blinded, background coloration was not considered to be a factor influencing chromatophore responses. Bioassays were generally conducted at the same time each day, during early afternoon hours, so that daily rhythms in coloration, which are known to occur in crustaceans (Rao, 1985), and indeed clearly observed in *G. lateralis* from Bimini by the second author (see Morphological Aspects section), should not have contributed to responses of the chromatophores. The following factors influencing chromatophorotropic activity in *G. lateralis* were examined.

1. Concentration of Eyestalk Extract. Dosage-response (or concentration-activity) curves for RPCH and BPDH were constructed with data derived from bioassays of extracts of fresh eyestalks made in heated seawater or perfusion fluid. Dosages ranged in concentration from 0.003 to 3.5 eyestalk-equivalents per 0.05 ml.

2. Proximity to Molt. In *G. lateralis,* a rapidly increasing R_3 value (R_3 = length of third limb bud divided by the width of the carapace × 100) indicates that a crab is approaching molt (see Bliss and Boyer, 1964; Bliss and Hopkins, 1974). To determine whether RPCH and BPDH activity are influenced by an animal's progression toward molt, destalked specimens of *G. lateralis* with R_3 values ranging from zero to 20 (approximately the beginning of terminal plateau, or about stage D_2 of Drach, 1939; see also Skinner, 1962; Tchernigovtzeff, 1972) were injected with eyestalk extract having a concentration of 0.3 ES/0.05 ml and observed for chromatophorotropic activity.

3. Physical and Chemical Treatments. Several physical and chemical tests (e.g., the effects of temperature, gel filtration, and solubility in various solvents) on eyestalk extracts were also conducted. However, the details on methodology will not be presented here; the results of these tests will be considered in brief in the Discussion section.

Results

Effect of Destalking and Injecting Eyestalk Extract: Upon removal of eyestalks, the melanophores and erythrophores in *G. lateralis* assume completely opposite states; i.e., the pigments within the melanophores concentrate maximally (stage 1), while the pigments within the erythrophores disperse maximally (stage 5). This has, very conveniently, allowed us to simultaneously study these two chromatophoral types in the same animal. For example, when crude eyestalk extract is injected into a destalked crab, melanophoral pigments disperse and erythrophoral pigments concentrate within their respective cells within minutes (fig. 7). The extent to which pigment concentration or dispersion occurs and the length of time that it persists after injection of extract depends upon the concentration of the extract and upon treatment of the extract prior to injection. These effects of crude extracts and other treatments on extracts and on individual crabs will be examined further in subsequent sections.

Chromatophorotropic Activity Within the Central Nervous System: With respect to RPCH, of the three central nervous system tissues examined, the eyestalks were found to contain most of the activity (fig. 8A, B). The thoracic ganglionic mass contained 45–52 percent of the activity found in the eyestalks, while the supraesophageal ganglion was more variable, registering 3 percent of the activity found in the eyestalks in Experiment A, and 42 percent in Experiment B.

When eyestalks, thoracic ganglionic mass, and supraesophageal ganglia were pooled in various combinations, extracted, and subsequently bioassayed, the total RPCH activity obtained appears to be considerably less in comparison with the total activities obtained from the bioassay of individual tissue extracts. For example, in Experiment A (fig.

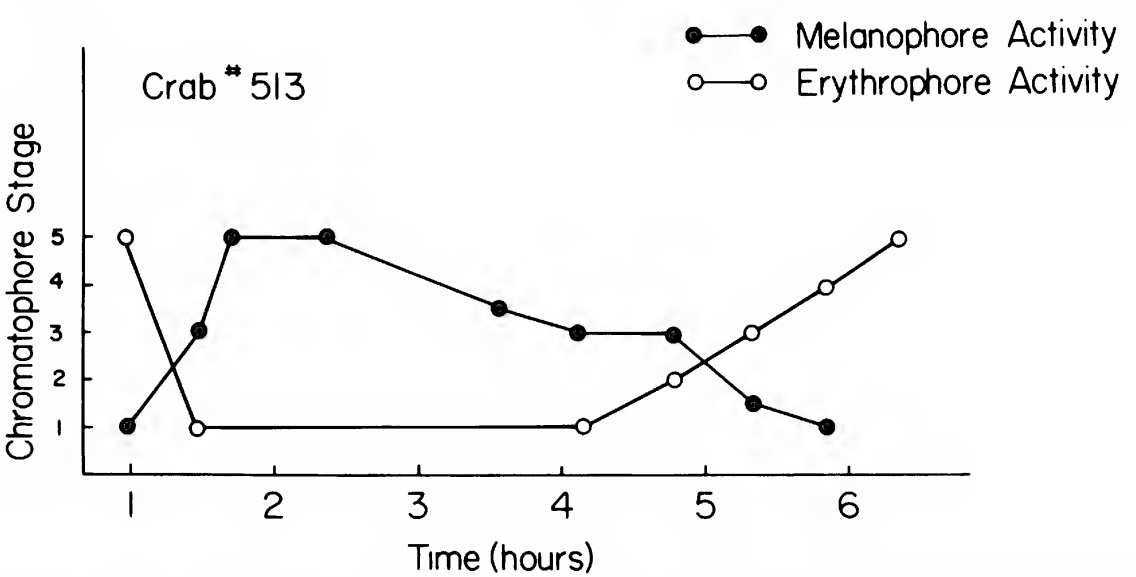


Fig. 7. Chromatophoral pigment migration in a destalked *Gecarcinus lateralis* in response to injection of aqueous extract of eyestalks. Concentration: 0.15 eyestalk equivalents.

8A), the amount of RPCH activity registered from the bioassay of extract prepared from pooled eyestalks, thoracic ganglionic mass, and supraesophageal ganglia (P_1) is less than the collective activities of the individually prepared and bioassayed tissue extracts.

In Experiment B (fig. 8B), combining the RPCH activities obtained from the bioassay of individual extracts of eyestalks and supraesophageal ganglia, or from eyestalks and thoracic ganglionic mass, the total is approximately 42–45 percent greater than that obtained from the bioassay of eyestalk extract alone. Yet, pooled extracts of eyestalks and supraesophageal ganglia (P_2) yielded a total RPCH activity of only 87 percent of that of eyestalks, while pooled extract of eyestalks and thoracic ganglionic mass (P_3) gave a total activity of 108 percent of eyestalks. The total RPCH activity obtained from separate extracts of supraesophageal ganglia and thoracic ganglionic mass tissues summed together appears to be just slightly less than that of the activity of eyestalks alone, while the pooled extract of supraesophageal ganglia and thoracic ganglionic mass tissues (P_4) yielded a total activity of only 65 percent of the eyestalks. These results suggest that a substance antagonistic to RPCH, possibly a red pigment-dispersing hormone (RPDH), or some inhibitory factor may exist within the supraesophageal ganglia and/or the thoracic ganglionic mass of *G. lateralis.*

Unlike the activity of RPCH, that of BPDH proved to be greater in the supraesophageal ganglia and thoracic ganglionic mass of *G. lateralis* than in the eyestalks (fig. 9A, B). The thoracic ganglionic mass contained 137–177 percent of the BPDH activity found in the eyestalks, while the supraesophageal ganglia contained 96 to 198 percent. As with RPCH activity, combinations of tissues or extracts of tissues contained less BPDH activity than that which would be obtained by simply summing the activities of the individual tissue extracts, again suggesting the presence of an opposing or inhibiting factor. In Experiment A (fig. 9A), for instance, the amount of BPDH activity in the extract of pooled eyestalks, supraesophageal ganglia, and thoracic ganglionic mass tissues (P_1) is much less than the amount of activity obtained from the total BPDH activities of individual tissue extracts.

In Experiment B (fig. 9B), the same was true; pooled supraesophageal ganglia and thoracic ganglionic mass tissue extracts (P_4) registered 327 percent of the BPDH activity of the eyestalks, whereas the total of all the individual BPDH activities obtained from each of the tissue extracts appeared to be much greater. However, extracts of pooled eyestalks and supraesophageal ganglia (P_2), and

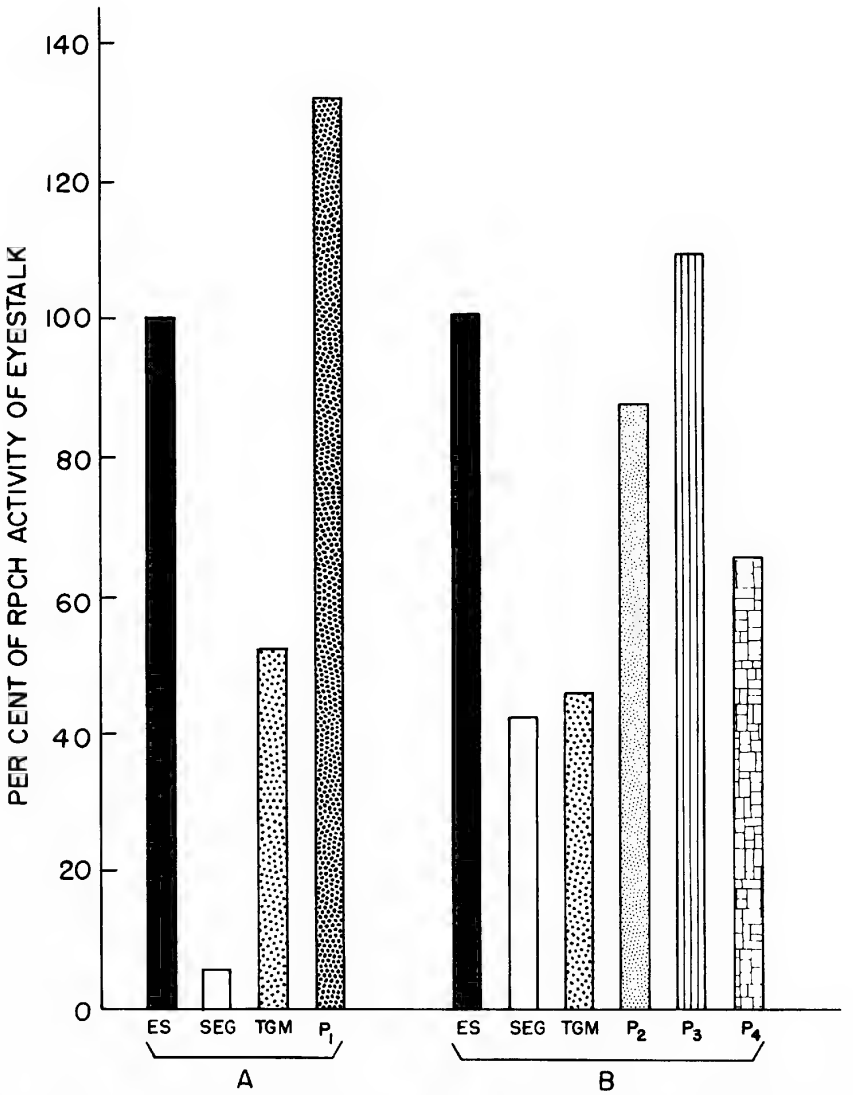


Fig. 8. Distribution of RPCH in eyestalks (ES), supraesophageal ganglia (SEG), and thoracic ganglionic mass (TGM) of *Gecarcinus lateralis*. Values are means, expressed as percent of that of ES. (Experiment A: one donor; ES = 0.1 eyestalk equivalents; SEG = 0.05 supraesophageal ganglia equivalents; TGM = 0.05 thoracic ganglionic mass equivalents; P_1 = pooled tissues, 0.1 ES, 0.05 SEG, and 0.05 TGM equivalents. Experiment B: six donors; ES, SEG, and TGM as in Experiment A; P_2 = pooled extracts of ES and SEG; P_3 = pooled extracts of ES and TGM; P_4 = pooled extracts of SEG and TGM)

pooled eyestalks and thoracic ganglionic mass tissues (P_3), exhibited more BPDH activity than the total amount of activity obtained from separate individual tissue extracts. Apparently, the inhibitory factor is manifested when preparations of supraesophageal ganglia and thoracic ganglionic mass tissues occur together.

Factors Affecting Chromatophorotropic Activity of Eyestalk Extract: 1. Concentration of Eyestalk Extracts. Dosage-response curves for RPCH and BPDH activity are presented in figure 10. The curves show a rise in chromatophorotropic activity with increasing concentration of eyestalk extracts until a maximum level is reached. This occurs at about 0.3 eyestalk equivalents per dose for RPCH and at about 0.7 eyestalk equivalents per dose for BPDH. Activity declines with additional concentration.

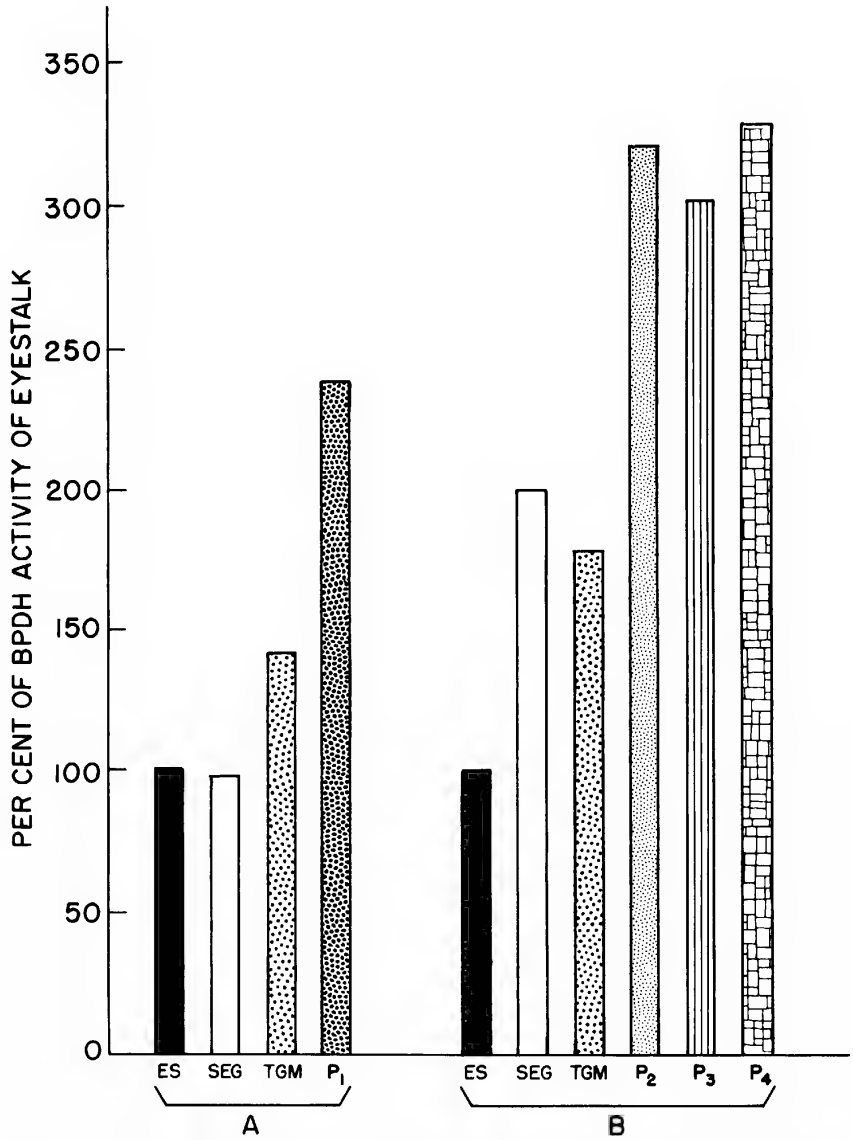


Fig. 9. Distribution of BPDH in eyestalks, supraesophageal ganglia, and thoracic ganglionic mass of *Gecarcinus lateralis*. Values are means, expressed as percent of that of ES. (Symbols as in fig. 8)

2. Proximity to Molt. No consistent increasing or decreasing trend is noted in the response of erythrophores when crabs are injected with eyestalk extract as a function of proecdysial stage (table 3). Erythrophores appear to be least responsive in crabs with low (0–4) R_3 values (19.5% less when compared with those of crabs having R_3 values of 12.1–16.0, the next highest RPCH activity recorded), and most responsive in crabs with R_3 values of 8.1–12.0. The differences in erythrophore responses among animals having R_3 values between 4.1 and 20.0 are not significant ($P > 0.05$), yet the difference in activity between a crab with an R_3 value of 0–4 and a crab with an R_3 value of 12.1–16.0 is ($P < 0.05$).

The response of melanophores, on the other hand, tends to increase with increasing R_3 values. However, the increased response is most significant ($P < 0.05$) when R_3 values reach 16.1–20.0 (36% over the next least recorded BPDH activity, or crabs at R_3 values of 8.1–12.0). At R_3 values greater than 20.0,

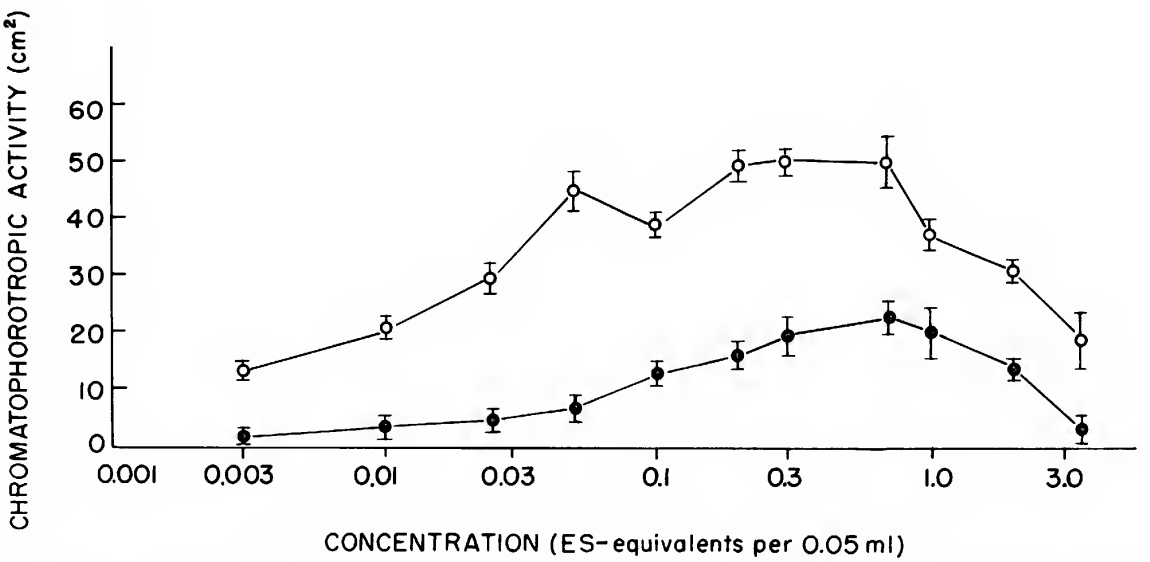


Fig. 10. Dosage-response curves of RPCH (O——O) and BPDH (●——●) of heated aqueous extracts of *Gecarcinus lateralis* eyestalks. Values are means ± SE, expressed in terms of area under curves.

observation of chromatophores becomes increasingly difficult due to the progressive separation of the epidermis from the exoskeleton.

GENERAL DISCUSSION

Although subspecies of *Gecarcinus lateralis* are not recognized, individuals from Bimini in the Bahamas and those from Bermuda differ markedly in coloration. In the former the legs and rear part of the carapace are a light to dark gray; in the latter those areas are variously bright orange, red, yellow, or light pink—but seldom gray. This difference in coloration is related in part to the number and degree of dispersion of the black and red chromatophore pigments within the epithelium, and in part to the pigment of the shell.

Local variations in color pattern of the prawn *Leander serratus* (reported by Carlisle, 1955) result from variations in the deposition and arrangement of the chromatophores that form the pattern of the cephalothoracic shield and the forward portion of the abdomen. Despite considerable individual variation, an overall difference in pattern exists between the prawns of Plymouth on the southern coast of England and those of Roscoff on the north coast of France. Carlisle interpreted these color differences as local variations resulting from a certain amount of geographic isolation and not sufficient to warrant subspecific recognition.

In this regard, the investigations of Boden (1952) on the conservation of insular plankton, specifically that of the Bermuda islands, are of interest relative to our findings on *G. lateralis*. During the summer the waters of the Bermudas have an anticyclonic circulation of thermal origin which tends to spread warm, highly saline water above and around the Bermuda platform until the water reaches a point of convergence, whereupon it becomes sufficiently cooled to sink and, partially mixed with ocean water, to return to the lagoon. Tows with a plankton net have shown plankton to be concentrated at the

TABLE 3
Chromatophorotropic Activity as a Function of Proximity to Molt

R values	N	Activity[a] RPCH	Activity[a] BPDH
0–4.0	8	46.6 ± 2.5	5.4 ± 1.6
4.1–8.0	20	69.3 ± 3.9	5.0 ± 1.0
8.1–12.0	8	74.7 ± 6.6	9.4 ± 3.4
12.1–16.0	11	57.9 ± 6.3	8.8 ± 1.2
16.1–20.0	15	61.8 ± 5.3	12.8 ± 2.3

[a] Mean ± SE; dosage = 0.3 eyestalk-equivalents/0.05 ml.

convergence. Thus there is a strong likelihood that the circulation of water in and around Bermuda during the summer may conserve the plankton of that area; or, in other words, the waters of the Bermudas may be repopulated by offspring of their own fauna. Since the larval stages of *G. lateralis,* as well as of other terrestrial crabs and of semiterrestrial and marine crabs, are free-swimming members of the oceanic plankton, the findings of Boden are of considerable significance in terms of the Bermuda brachyuran fauna.

Earlier, Verrill (1908) suggested that the decapod crustacean fauna of Bermuda originated in the West Indies, particularly in the Bahamas, and that the free-swimming larval forms were carried northward by the Gulf Stream (Florida Current) and the prevailing wind currents. There is nothing to argue against this view, except perhaps in the case of some forms where the larval existence may be too short in duration for these plankton to reach Bermuda. However, with reference to the general marine populations, it has long been recognized that Bermudan and West Indies species are closely related. Evidence for this was provided by R. Tucker-Abbott and co-workers (personal commun.) at the Academy of Natural Sciences in Philadelphia. They noted that within approximately the last 20 years, many species of mollusks, which were previously rare or absent, have become fairly common in the waters of Bermuda. The large calico clam, *Macrocallista maculata,* for example, was caught alive for the first time in 1961. By 1965 this species became so abundant that it was being used as bait by fishermen and was appearing in the local Bermudan fish markets. Tucker-Abbott and co-workers concluded that an invasion into the waters of Bermuda is occurring, probably from the waters of Florida or the Bahamas or both. They attribute the invasion to the transport both of planktonic larvae via the Gulf Stream system and of living ovigerous adults in the bilgewater of leaky sailboats arriving in Bermuda from Florida and the Bahamas. Significantly, many of the newly conspicuous Bermudan mollusks are also abundant in the Bahamas, Cuba, and the outer Florida Keys.

Although it is possible that the decapod crustacean fauna of Bermuda may continually be enriched by an influx of West Indian individuals, nevertheless, Boden's findings suggest that a substantial proportion may result from repopulation by its own young. It is conceivable, therefore, that individuals of *G. lateralis* now living in Bermuda are largely descendents of earlier inhabitants of the same islands rather than of more recent arrivals from the Bahamas and other parts of the West Indies. It is also conceivable that, as a result, there may have developed in Bermuda a separate geographical race of these crabs, superficially distinguishable by coloration.

The adaptiveness of color change among Crustacea may well be related to antipredation strategies as well as to social signaling (DeCoursey, 1983). Powers and Bliss (1983), though, suggested that among gecarcinids the striking color patterns may function more in camouflage or disruptive coloration than as social signaling devices.

In *G. lateralis,* pigment granules within the epithelial chromatophores contribute to total coloration. There are significantly more melanophores and significantly fewer erythrophores in the posterior and dorsolateral parts of the carapace of crabs from Bimini than of crabs from Bermuda (see table 2). The differences may well be related to their respective habitats. In Bimini, the soils are predominantly gray and crab-eating predators, notably herons and bitterns, are numerous (Bliss, 1979). Conversely, in Bermuda, pink and white coral sand and brightly colored flowers prevail, while avian predators are relatively few. Therefore, in this species, selection pressure may have favored the development of a separate color variety in each geographic area.

Quantitative studies based on amplitude and duration of chromatophore responses in relation to background coloration and various stimuli, including illumination and temperature, have been conducted on several species of crustaceans (Sandeen, 1950; Fingerman et al., 1967; Rao, 1985). However, except for the blanching responses of *Uca* at high temperatures (Smith and Miller, 1973), little work has been done on the ecological and behavioral aspects of color change, although circadian rhythms of color change have been noted in *Carcinus* (Powell, 1962) and in *Uca* (Stephens, 1962).

Physiologically, there is no evidence that populations of *G. lateralis* from Bimini and Bermuda differ. In crabs from both areas, removal of eyestalks causes the pigment within the erythrophores to disperse and that within the melanophores to concentrate. Injection of eyestalk extract into destalked individuals has the opposite effect, i.e., the dispersed red pigment concentrates and the concentrated black pigment disperses (fig. 7).

As in other crustaceans, the hormones that control pigment migration within the chromatophores of *G. lateralis* are produced in various components of the central nervous system. These are stored in and eventually released from the sinus gland located in the eyestalks. The fact that both erythrophores and melanophores are present in this species, and the fact that the respective pigments migrate in opposite directions when bioassays are conducted, has permitted us to simultaneously study the responses of the two chromatophoral types as well as some of the physical and chemical characteristics of the respective chromatophorotropins.

The phenomenon of a dual and antagonistic response of red and black chromatophores is not unique to *G. lateralis.* It is found in several other species, e.g., in the freshwater crab *Eriocheir japonicus* (Matsumoto, 1954), the blue crab *Callinectes sapidus* (Fingerman, 1956), the green crab *Carcinus maenas* (Powell, 1962), the ghost crab *Ocypode macrocera* (Rao, 1967a, 1967b), and the mud crab *Rhithropanopeus harrisi* (Skorkowski, 1972). In the fiddler crab, *Uca pugilator,* both red and black chromatophores are also present, but the pigments do not migrate in opposite directions. Instead, they either assume fully concentrated states when the crabs are destalked, or they disperse when the crabs are injected with extract (Fingerman, 1968).

As has been firmly established with other crustaceans (see Rao, 1985; Newcomb et al., 1985), the chromatophorotropins RPCH and BPDH in *G. lateralis* are undoubtedly peptides. Both molecules are subject to endogenous enzymatic action, and the nonspecific proteolytic enzyme Pronase destroys each one within 3–3.5 hours (unpubl. data). Furthermore, the differences in molecular composition between RPCH and BPDH can be seen by the fact that trypsin and chymotrypsin totally destroy BPDH activity, but only reduce that of RPCH.

The dosage-response curves presented in figure 10 for *G. lateralis* eyestalk extracts suggest a physiological limit beyond which further increases in concentration do not evoke greater chromatophore responses. Indeed, after a certain point, the curves show a decrease in chromatophorotropic response with increasing concentration of eyestalk extract. A similar phenomenon was first noted by Sandeen (1950) with eyestalk extracts of *U. pugilator* and later by Fingerman et al. (1964) with eyestalk extracts of *U. pugnax.* Possibly, the decrease in chromatophorotropic activity with increasing concentration of eyestalk extract could be due to an accompanying increase in antagonistic factors within the extracts. Our experiments with pooled extracts of various tissues suggest the existence of such factors within the central nervous system of *G. lateralis.* Thus, less chromatophorotropic activity was obtained when aqueous extracts of eyestalks, supraesophageal ganglia, and thoracic ganglionic mass were pooled than when the activities of the individual tissue extracts were summed.

Mutually antagonistic hormones operating on specific chromatophores and identified from eyestalk and extraeyestalk nervous tissues have been shown to exist in a number of other crustaceans, including *Crangon* (Brown, 1952; Fingerman and Fingerman, 1972; Skorkowski, 1971), *Palaemonetes* (Brown et al., 1952; Fingerman and Couch, 1967), *Palaemon* (Knowles et al., 1955), and *Ocypode* (Rao, 1967a). Investigations by Rao et al. (1967a), for example, reveal the presence of white pigment-dispersing (WPDH) and white pigment-concentrating (WPCH) substances in *U. pugilator.* The circumesophageal connectives were shown to contain BPDH and WPCH, while the supraesophageal ganglia contained WPDH in addition to BPDH. In another study, Rao et al. (1967b) obtained increased melanin-dispersing activity by filtering the extracts through a column of Bio-Gel P-2. Presumably, melanin-concentrating hormone was retained by the column.

When aqueous extracts of *G. lateralis* eyestalks are first filtered through a column of Sephadex G-25 (or when the pigmentary ef-

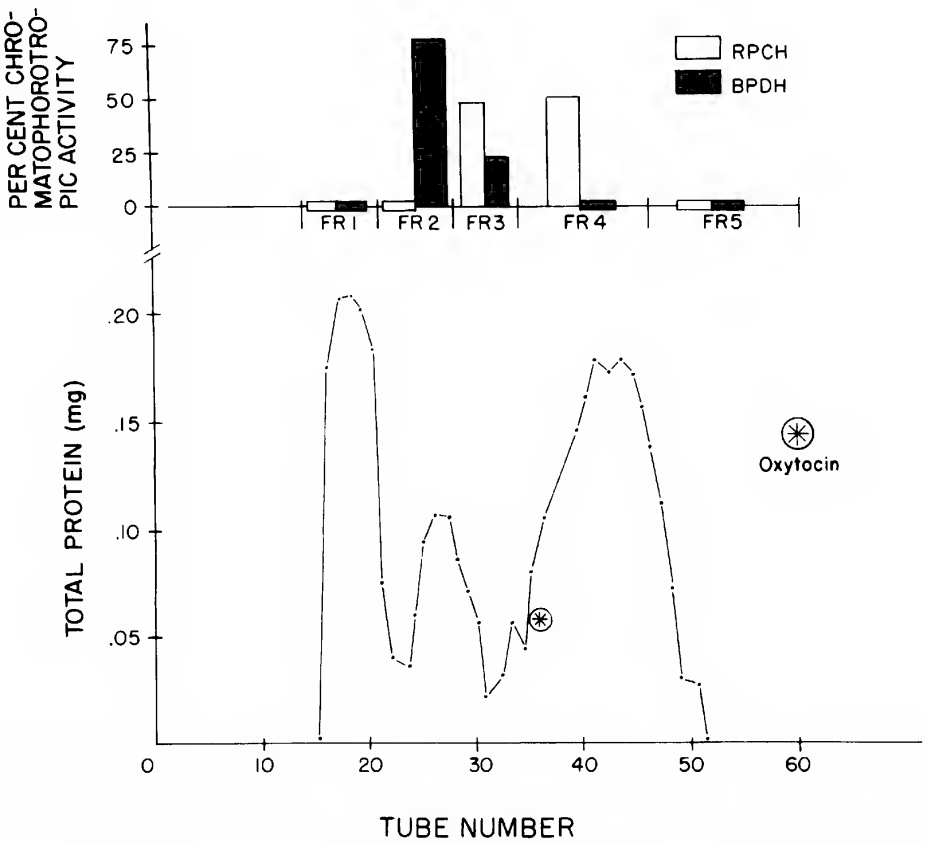


Fig. 11. Elution profile and chromatophorotropic activity of fractions (FR) eluted from a Sephadex G-25 column. A total of 279 lyophilized eyestalks were processed. The tested fractions (1–5) represent approximately 10 eyestalk equivalents.

fector hormones are initially extracted with ethanol; unpubl. data), a dramatic increase in the activity of both RPCH and BPDH is obtained upon assaying the appropriate fractions (see fig. 11). Curves representing the total chromatophorotropic activity obtained from the Sephadex G-25 fractionated extracts are presented in figure 12. For comparison, they are plotted with those obtained from the bioassays of unfiltered aqueous extracts (dosage-response curves of fig. 10). Apparently, a phenomenon similar to that obtained by Rao et al. (1967b) with *U. pugilator* is also seen here; i.e., the retention within the Sephadex G-25 column of the antagonistic factors to both RPCH and BPDH, factors that are usually present in aqueous extracts. Thus, the assays of activity in the crude aqueous extracts were undoubtedly affected by the presence of antagonistic factors. Hence, the values given for the dosage-response curves (fig. 10) do not truly reflect the amounts of black pigment-dispersing and red pigment-concentrating hormones.

Recent investigations demonstrate that release of chromatophorotropins from the neurosecretory system is under the control of certain neurotransmitters (Fingerman, 1985, 1987; Rao, 1985). In *Uca,* for example, the putative neurotransmitter, 5-HT (serotonin) triggers the release of RPCH (Fingerman et al., 1981; Hanumante and Fingerman, 1982), while GABA (gamma-aminobutyric acid) was found to inhibit the release of BPDH in intact and isolated eyestalks (Quackenbush and Fingerman, 1984a). Additionally, octopamine was found to block the dispersion of black pigments, and met-enkephalin was shown to cause pigment concentration in both red and black chromatophores in *Uca* (Quackenbush and Fingerman, 1984a).

With respect to *G. lateralis,* Martinez et al. (1986) provided evidence for the involvement of opiatelike peptidergic substances and

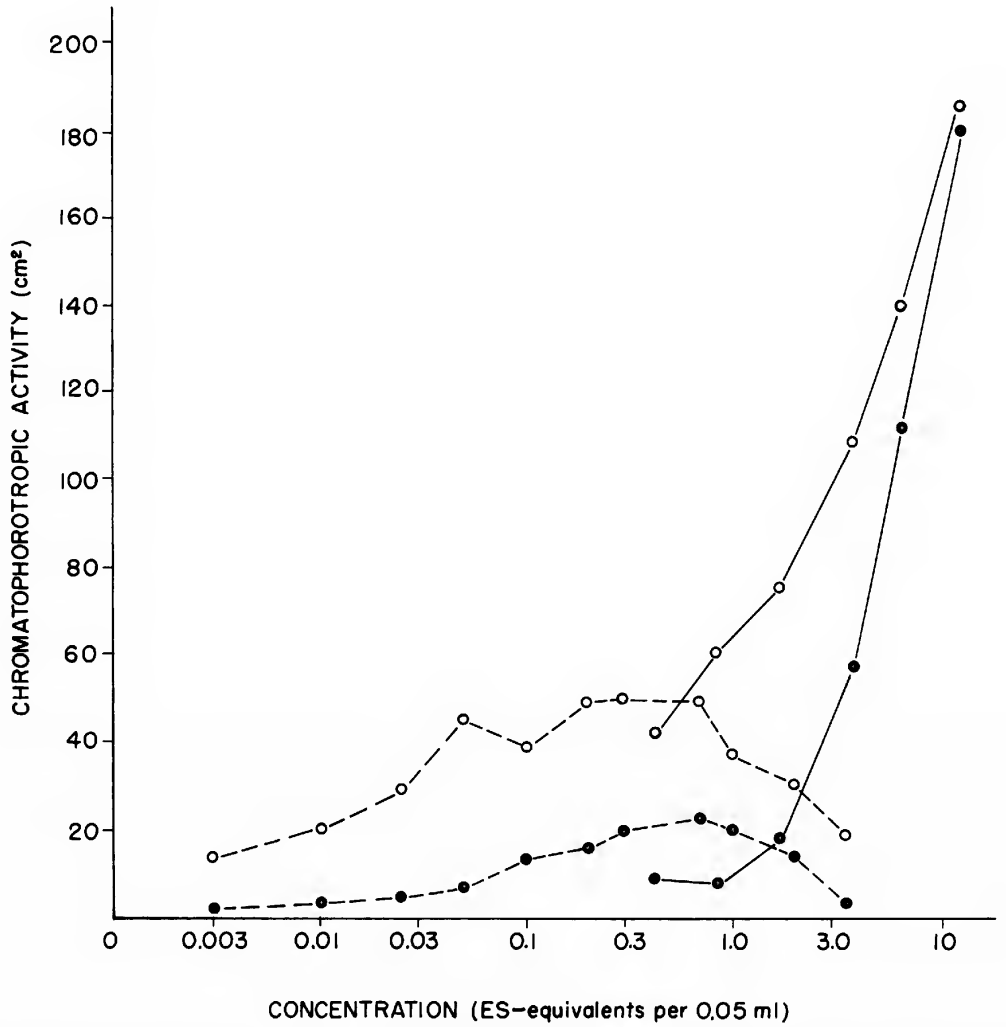


Fig. 12. Curves of activity of RPCH (○——○) and BPDH (●——●) obtained from fractions 2–4. For purposes of comparison, these curves are superimposed over the dosage-response curves of figure 10.

monoamines in the color change mechanism of this species. Interestingly, the action of these substances on *G. lateralis* differs in some ways from that on *U. pugilator.* For instance, dopamine does not seem to affect pigment dispersion in melanophores of *U. pugilator,* although it does stimulate the release of RPCH and BPCH (black pigment-concentrating hormone) (Fingerman, 1987; Quackenbush and Fingerman, 1984b). In *G. lateralis* not only does dopamine affect the release of RPCH, it also exerts a strong stimulating effect in the release of BPDH. In *Uca,* norepinephrine produces dispersion of melanophore pigments (Fingerman et al., 1981); however, in *G. lateralis,* this neurotransmitter was found to be without action on the movement of either red or black chromatophoral pigments. Furthermore, 5-HT elicited only erythrophore pigment dispersion in *Uca* (Fingerman and Fingerman, 1975) and it stimulated the release of BPDH in *Carcinus maenas* (Rao and Fingerman, 1970). In *G. lateralis* serotonin produced only a moderate migration of melanophore pigments and had no influence on the movement of erythrophore pigments.

With the opioid met-enkephalin, the results were interesting and complex. This substance had no effect in *G. lateralis,* although, as stated above, it was found to promote the concentration of red and black pigments in

Uca. Since this substance is easily degradable, and hence subject to decomposition in the presence of peptidases normally occurring in the crab, further tests were conducted with the highly stable analog of met-enkephalin, FK 33-824 (Martinez et al., 1986). This peptide was found to influence color change activity only when it was coinjected with eyestalk extract. In this manner it potentiated the stimulatory effect of the extract on both melanophore and erythrophore activity. It is important to note also that the effect of FK 33-824 was completely blocked by prior treatment with naloxone, thus demonstrating stereoselectivity in its mechanism of action.

Preliminary binding experiments indicate that peripheral opioid binding sites are absent from the pigmented hypodermis of *G. lateralis*. Thus, any effects that opioids may have on the influence of chromatophorotropic mechanisms appear to be centrally mediated. Of interest is the detection of enkephalin-like substances in the eyestalk and brain of this species (Leung et al., 1987).

Variations in the actions of signal molecules among the species cited above may be of evolutionary significance in that various species of crabs appear to use the same molecules but to different ends. The exact significance of this phenomenon is presently not known; however, it may be related to a particular organism's immediate environmental requirements.

The extent and precise nature of the neurotransmitter-mediated hormone release mechanism as it relates to color change among crustaceans and its evolutionary significance will continue to be of interest to a number of investigators. In the meantime, the observations presented in this paper on the morphological and physiological aspects of coloration in *G. lateralis* provide additional information regarding the biology of this remarkable, terrestrially adapted crab.

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